

LISTING OF CLAIMS

- 1–31. (Cancelled)
32. (Currently amended) A microfluidic device, comprising:
a microfluidic network, wherein the microfluidic network comprises several modules, including:
 an enrichment module configured to accept a particle-containing fluid sample, and to create an enriched sample therefrom;
 a microdroplet preparation module configured to receive the enriched sample and to create a microdroplet therefrom;
 a cell lysing module configured to lyse the microdroplet of enriched sample;
 a mixing module configured to mix one or more reagents and the enriched sample; and
 a DNA manipulation module configured to receive the enriched sample mixed with one or more reagents;
wherein the several modules are operatively connected to one another by one or more channels; and wherein at least one of the several modules is isolatable by a valve; and wherein at least one actuator is configured to move a microdroplet of fluid from one of the several modules to another of the several modules.
33. (Previously presented) The microfluidic device of claim 32, further comprising:
a lower substrate having a base and an oxide layer, wherein the oxide layer contains a plurality of resistive heaters; and
an upper substrate having a bottom surface, wherein the bottom surface is bonded to the oxide layer on the lower substrate, and wherein the bottom surface includes the microfluidic network.

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34. (Previously presented) The microfluidic device of claim 33, wherein the base is made of glass.
35. (Previously presented) The microfluidic device of claim 33, wherein the upper substrate is composed of a material selected from the group consisting of: glass, silicon, ceramic, plastic, and quartz.
36. (Previously presented) The microfluidic device of claim 33, wherein the silicon oxide layer prevents each of the plurality of resistive heaters from directly contacting the fluid in the microfluidic network.
37. (Previously presented) The microfluidic device of claim 32, wherein at least one of the one or more channels has a micro-scale dimension.
38. (Previously presented) The microfluidic device of claim 37 wherein the microscale dimension is less than 75 μm .
39. (Previously presented) The microfluidic device of claim 37 wherein the microscale dimension is less than 250 μm .
40. (Previously presented) The microfluidic device of claim 33, wherein each heater of the plurality of resistive heaters is connected via electric leads to a pair of terminals exposed at the edge of the lower substrate.
41. (Currently amended) The microfluidic device of claim 40, further comprising configured to accept electrical control signals from a digital data acquisition and control board, wherein the control board is electrically connected to the terminals.

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42. (Currently amended) The microfluidic device of claim 41 further comprising a computer, wherein the computer is configured to provide instructions to wherein the data acquisition and control board is configured to receive instructions from a computer to apply a voltage across the pair of terminals and to cause a current to flow through the electric leads, thereby activating one or more of the resistive heater heaters.
43. (Previously presented) The microfluidic device of claim 32, further comprising a sample input module, and a reagent input module, respectively configured to permit sample and reagent materials to be introduced into the device.
44. (Previously presented) The microfluidic device of claim 43, wherein at least one of the sample input module and the reagent input module is configured to accept material input from a computer controlled laboratory robot.
45. (Currently amended) The microfluidic device of claim [[43,]] 32, wherein the sample is a fluid containing whole cells.
46. (Previously presented) The microfluidic device of claim 45, wherein the cells in the sample are bacteria, animal or human cells.
47. (Currently amended) The microfluidic device of claim [[43,]] 32, wherein the sample comprises intracellular material.
48. (Currently amended) The microfluidic device of claim 32, further comprising an input module, wherein the enrichment module is configured to accept the particle-containing fluid sample from the input module.
49. (Currently amended) The microfluidic device of claim 48, wherein the enrichment module is configured to concentrate particles in the particle-containing fluid sample, thereby

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creating an enriched sample having a substantially higher ratio of particles per volume of fluid than a corresponding ratio of the particle-containing fluid sample.

50. (Currently amended) The microfluidic device of claim 32, wherein the enrichment module comprises:

a flow-through member;
an enrichment chamber adjacent the flow-through member;
a downstream channel connecting the flow-through member to the microdroplet preparation module;
an actuator configured to force enriched sample from the enrichment chamber to the downstream channel;
a valve disposed between the actuator and the flow-through member; and
a valve disposed between the flow-through member and the downstream channel,
wherein the enrichment module is configured to allow fluid of a particle-containing fluid to pass through the flow-through member thereby accumulating the enriched sample,
comprising particles of the particle-containing fluid, in the enrichment chamber.

51. (Currently amended) The microfluidic device of claim 50, wherein the flow-through member comprises:

a first surface adjacent the enrichment chamber; and
a second surface spaced apart from the enrichment chamber and adjacent a self-contained space, wherein the space contains an absorbent material.

52. (Previously presented) The microfluidic device of claim 50, wherein the flow-through member comprises a material having pathways smaller than the diameter of particles in the sample to be enriched.

53. (Previously presented) The microfluidic device of claim 50, wherein the actuator is a thermally actuated gas actuator.

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54. (Currently amended) The microfluidic device of claim 53, wherein the thermally actuated gas actuator comprises a resistive heater of the plurality of resistive heaters located beneath a chamber in the microfluidic network.

55. (Previously presented) The microfluidic device of claim 54, wherein the resistive heater is in thermal contact with the chamber, and wherein the chamber contains a volume of gas.

56. (Previously presented) The microfluidic device of claim 53, wherein the actuator is integral with the upper substrate.

57. (Previously presented) The microfluidic device of claim 32, wherein the microdroplet preparation module comprises:

- a channel;
- a positioning element situated in the channel;
- a valve in communication with the channel; and
- a gas actuator in communication with the channel, and configured to split a microdroplet from a fluid sample situated in the channel.

58. (Previously presented) The microfluidic device of claim 57, wherein the positioning element is a non-wetting positioning element, or an active fluid positioning element.

59. (Currently amended) The microfluidic device of claim 32, wherein the microdroplet preparation module is configured to accept enriched sample from the enrichment module, and is further configured to send a microdroplet of enriched sample to the cell lysing module.

60. (Previously presented) The microfluidic device of claim 32, wherein the cell lysing module comprises:

- a lysing zone;
- a lysing mechanism within the lysing zone; and

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a positioning element positioned upstream from the lysing zone.

61. (Previously presented) The microfluidic device of claim 60, wherein the positioning element is selected from the group consisting of: a non-wetting positioning element; a capillary-assisted positioning element; a vented positioning element; and an active fluid positioning element.

62. (Previously presented) The microfluidic device of claim 60, wherein the lysing mechanism comprises:

one or more electrodes that communicate with a digital acquisition and control board, and configured to deliver a pulsed electric field to a microdroplet containing cells, wherein the microdroplet is in contact with the electrodes.

63. (Currently amended) The microfluidic device of claim 32, wherein the cell lysing module is configured to accept a microdroplet of enriched sample from the enrichment module, and is further configured to deliver a lysed microdroplet to the mixing module.

64. (Previously presented) The microfluidic device of claim 32, wherein the cell lysing module comprises:

a lysing zone;
a lysing mechanism within the lysing zone;
an actuator disposed upstream of the lysing zone; and
a positioning element positioned downstream from the lysing zone.

65. (Previously presented) The microfluidic device of claim 32, wherein the mixing module comprises:

a first channel; and
a second channel, adjoined to the first channel.

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66. (Previously presented) The microfluidic device of claim 65, wherein the first channel is configured to accept a lysed microdroplet from the microdroplet preparation module.
67. (Currently amended) The microfluidic device of claim 66, wherein the sample comprises intracellular material or whole cells, and wherein the lysed microdroplet comprises polynucleotides.
68. (Previously presented) The microfluidic device of claim 65, further comprising:
 - a reagent input module;
 - wherein the second channel of the mixing module is configured to accept reagent from the reagent input module.
69. (Previously presented) The microfluidic device of claim 68, wherein the reagent input module comprises:
 - a reagent source channel, in communication with the second channel;
 - an input port configured to accept reagent, and to deliver a microdroplet of the reagent to the reagent source channel;
 - a valve in communication with the reagent source channel;
 - a gas actuator in communication with the reagent source channel via an opening, and configured to split the microdroplet from the reagent when situated in the reagent source channel; and
 - a positioning element situated in the reagent source channel downstream from the actuator.
70. (Previously presented) The microfluidic device of claim 69, wherein the reagent input module further comprises an overflow channel adjoining the reagent source channel.

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71. (Previously presented) The microfluidic device of claim 69, wherein the gas actuator in communication with the reagent source channel includes a second positioning element configured to prevent reagent from entering.
72. (Previously presented) The microfluidic device of claim 69, wherein the opening and the positioning element are situated at a distance from one another that ensures that the microdroplet of reagent has a predetermined volume suitable for mixing with a lysed microdroplet of sample.
73. (Previously presented) The microfluidic device of claim 69, wherein the positioning element is selected from a group consisting of: a non-wetting positioning element; a capillary-assisted positioning element; a vented positioning element; and an active fluid positioning element.
74. (Currently amended) The microfluidic device of claim 32, wherein the DNA manipulation module comprises:
- a DNA manipulation zone configured to accept a microdroplet of mixed lysed sample and reagent from the mixing zone module;
 - a vent configured to prevent pressure from increasing in the DNA manipulation zone during introduction of the mixed lysed sample reagent therein;
 - a first valve; and
 - a second valve;
 - wherein the first and second valves are positioned to prevent materials from exiting the DNA manipulation zone, when closed, and wherein the DNA manipulation zone is configured with heat sources.
75. (Previously presented) The microfluidic device of claim 74, further comprising a detector in communication with DNA manipulation zone.

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76. (Currently amended) The microfluidic device of claim 74, wherein the DNA manipulation module is configured to carry out one or more of restriction, digestion, ligation, hybridization, or amplification of DNA material in the microdroplet of mixed lysed sample and reagent.
77. (Currently amended) The microfluidic device of claim [[76,]] 74, wherein the heat sources are configured under control of computer to allow thermal cycling of the DNA manipulation zone.
78. (Currently amended) The microfluidic device of claim [[76,]] 74, further comprising a light source that introduces light into the DNA manipulation zone.
79. (New) The microfluidic device of claim 33 wherein both the lower substrate and the upper substrate are composed of plastic.
80. (New) the microfluidic device of claim 79, wherein the upper substrate and microfluidic network are formed by injection moulding.
81. (New) The microfluidic device of claim 49, whercin the ratio of particles per volume of fluid in the enriched sample is about 25 times higher than the ratio of particles per volume of fluid in the particle-containing fluid sample.
82. (New) The microfluidic device of claim 49, wherein the ratio of particles per volume of fluid in the enriched sample is about 250 times higher than the ratio of particles per volume of fluid in the particle-containing fluid sample.
83. (New) The microfluidic device of claim 49, wherein the ratio of particles per volume of fluid in the enriched sample is about 1,000 times higher than the ratio of particles per volume of fluid in the particle-containing fluid sample.

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84. (New) A microfluidic cartridge containing the microfluidic device of claim 1.